IN THE CLAIMS

1. (Twice Amended) A plurality of single-stranded oligonucleotide DNA primers for simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a multiplex polymerase chain reaction (PCR), said primers having a 5' domain, X, and a 3' domain, Y, wherein

a) said 5'-X domains each comprise a common sequence that does not hybridize to said multiple target sequences, said common sequence not comprising a restriction enzyme recognition site sequence;

b) the melting temperature of a hybrid between X and its complement in the absence of other sequences is greater than about 60°C;

c) said 3'-Y domains each comprise a unique sequence contained within or flanking one of said multiple target sequences or its complement; [and]

d) the melting temperature of a hybrid between at least one of said 3'-Y domains and its complement, in the absence of other sequences, is different from the melting temperature of a hybrid between at least one other 3'-Y domain and its complement present in said multiplex PCR; and

each of said primers being capable of annealing specifically with its cognate target sequence under uniform high stringency annealing conditions during said amplification.

2.(Reiterated) The primers according to claim 1, wherein X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64).

3.(Reiterated) The primers according to claim 1, wherein X and Y each comprise from 17 to 20 bases.

4.(Reiterated) The primers according to claim 1, wherein the melting temperature of a hybrid formed between each of said primers and its complement in a solution of 0.5M NaCl is at least 72°C.

5.(Reiterated) DNA primers for simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a multiplex polymerase chain

reaction (PCR), wherein said primers consist of the sequence 5'-GCGGTCCCAAAAGGGTCGT (SEQ ID NO:64) (Y)-3', wherein an individual Y comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement.

6. (Reiterated) A method for simultaneous amplification of multiple DNA target sequences present in a DNA sample, said method comprising:

a) contacting said DNA sample, in a single reaction mixture, with a multiplicity of paired oligonucleotide primers having structure 5'-XY-3', wherein

(i) each X comprises the sequence 5'- GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and

(ii) each Y comprises a unique sequence contained within or flanking one of said multiple target sequences or its complement; and

b) performing multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters.

7.(Reiterated) A method for simultaneously detecting the presence of multiple defined target DNA sequences in a DNA sample, which comprises the steps of:

a) simultaneously contacting said DNA sample, in a single reaction mixture, with a multiplicity of oligonucleotide pairs, each of said pairs consisting of a first and a second oligonucleotide primer, wherein

(i) said first primer of each pair has the structure 5'-XY-3', wherein each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and each Y comprises a unique sequence contained within one of said multiple target sequences or its complement, and

(ii) said second primer of each pair has a structure 5'-XY-3', wherein each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and each Y comprises a unique sequence flanking one of said multiple target sequences or its complement;

b) performing multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters to form amplification products for each of said multiple defined target DNA sequences primed with said oligonucleotide; and

c) detecting the amplification products.

8.(Reiterated) The method of claim 7 wherein detection of amplification product indicates the presence of the target sequence in a DNA sample.

9.(Reiterated) The method of claim 7 wherein said detecting step comprises gel electrophoresis.

10.(Reiterated) A method for high-throughput genetic screening to simultaneously detect the presence of multiple defined target sequences in DNA samples obtained from one or more individuals, said methods comprising steps of:

a) providing a sample of DNA from said individual(s);

b) simultaneously contacting said DNA sample(s) with a multiplicity of oligonucleotide pairs, each of said pairs consisting of a first and second oligonucleotide primer, wherein

(i) said first primer of each pair has structure 5'-XY-3', wherein each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and each Y comprises a unique sequence contained within one of said multiple target sequences or its complement, and

(ii) said second primer of each pair has the structure 5'-XY-3', wherein each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and each Y comprises a unique sequence flanking one of said multiple target sequences or its complement;

c) subjecting said sample to multiple cycles of melting, reannealing, and DNA synthesis wherein each of said cycles is conducted under the same reaction conditions and cycling parameters to form amplification products for each of said multiple defined target DNA sequences primed with said oligonucleotides; and

d) detecting the amplification products.

11.(Reiterated) The method of claim 10 wherein detection of an amplification product indicates the presence of the target sequence in the DNA sample.

12.(Reiterated) The method of claim 10 wherein said detecting step comprises gel electrophoresis.

13.(Amended) A method for simultaneously amplifying and detecting multiple defined target sequences in a DNA sample, said method comprising the steps of:

a) simultaneously contacting said sample with a plurality of oligonucleotide pairs, each of said pairs consisting of a first and a second primer having the structure 5'-XY-3', wherein

(i) X in said first primer of each pair comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64) and Y comprises a unique sequence contained within one of said multiple target sequences or its complement, and

(ii) said second primer of each pair has the structure 5'-XY-3', wherein each X comprises the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64), and each Y comprises a unique sequence flanking one of said multiple target sequences or its complement;

c) subjecting said sample to multiple cycles of melting, reannealing, and DNA synthesis wherein each of said cycles is conducted under the same conditions and cycling parameters to form amplification products for each of said multiple defined target sequences primed with said oligonucleotides, and

d) detecting the amplification products.

14.(Amended) A method of screening to simultaneously amplify and detect multiple target sequences of interest in DNA, the method comprising:

a) obtaining a sample of DNA to be screened for said multiple target sequences of interest,

b) contacting said sample with a plurality of oligonucleotide primer pairs having the structure 5'-XY-3' under multiplex polymerase chain reaction conditions wherein coamplification of multiple target sequences occurs in one or more cycles of identical melting, annealing and extending temperatures and times, wherein

each 5'-X domain comprises a common oligonucleotide that is neither complementary nor specific for said multiple target sequences, said common oligonucleotide not comprising a restriction enzyme recognition site sequence; and

each 3'-Y domain comprises a unique oligonucleotide, each oligonucleotide complementary to and specific for one of said multiple target sequences of interest suspected to be present in said DNA; and

c) detecting the amplification products.

15.(Reiterated) A method according to claim 14, wherein said multiple target sequences of interest are located within different regions of a gene present in said DNA.

16.(Reiterated) A method according to claim 14, wherein said multiple target sequences of interest are located within ~~multiple genes present in said DNA.~~

17.(Amended) A plurality of amplified target sequences ~~of interest~~ amplified and detected according to the method of claim 13.

18.(Amended) A plurality ~~of~~ amplified target sequences of interest amplified and detected according to the ~~method~~ of claim 14.

REMARKS

The Claimed Invention

This invention relates to oligonucleotide primers for simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a multiplex polymerase chain reaction. The claimed invention is also directed to methods of simultaneous amplification and detection of multiple DNA target sequences present in a DNA sample. The claimed invention also relates to a method for high-throughput genetic screening and to a method of screening to simultaneously amplify and detect multiple target sequences of interest in DNA. The invention is directed, as well, to a plurality of amplified target sequences of interest amplified and detected according to the method.